

Research Article

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## Effect of low cost medium supplemented on micropropagation of banana (*Musa paradisiaca* L.) var. Poovan

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### Abstract

Banana is nutritionally significant, important food crops, which is widely grown and consumed throughout the world. The most widely used MS medium (Murashige and Skoog, 1962) is used for commercial production of plantlets through shoot-apical meristem culture of banana. High cost of plantlet production through micropropagation technique is a major concern limiting its wide application, despite its obvious advantages. The low cost medium was standardize using several low cost alternatives viz. macro, micro, iron, vitamin, solidifying agent, carbon source and growth regulators for the production of banana sword sucker explants. The LBTM supplemented with different concentration of growth regulators the high shoot induction frequency was obtained in the combination of BAP 0.2 mg/l + IAA 0.1 mg/l (65.6±6.2) followed by KIN 0.2 mg/l + IAA 0.1 mg/l (60.6±6.8) respectively. Multiple shoot induction was achieved in BAP 2.0 mg/l + IAA 0.5mg/l LBTM combination produced high frequency of shoots (18.3±3.7) followed by the combination of KIN 2.0 mg/l + IBA 0.5 mg/l (17.4±4.4) respectively, in root induction of IBA + GA<sub>3</sub> LBTM combination in this 2.0 mg/l IBA + 0.5 mg/l GA<sub>3</sub> produced more number of roots (10.9 ± 2.6) followed by other combination. In all the IBA combination root was significantly produced in sub cultured banana shoot tips var. poovan. The well developed shoots were transferred to hardening. The successful *ex vitro* plantlets are maintained in different potting mix for banana propagation.

### Keywords

MS medium,  
micro-propagation,  
variety,  
banana,  
Hardening

### Introduction

Banana is the fourth most important food crop in the world as well as in India (Ganapathi *et al.*,1999). It is a staple food and export commodity. It contributes to the food security of millions of people in the developing world and, when traded in local markets, provides income and employment to rural populations.

Micropropagation of banana is highly efficient allowing a large turnover of plants in a very short period of time with in a very little space (Arias, 1992; Arvanitoyannis

*et al.*, 2007). Commercial production of micropropagated banana is now common in many countries and it is estimated that 25 million plants are produced worldwide each year. Micropropagation techniques were established for fast multiplication of banana (Vuylsteke, 1989). One of the most important factors governing the *in vitro* shoot multiplication is largely determined by the composition of the culture medium (Rashid *et al.*, 2000). Murashige and Skoog (1962) medium is widely used for banana propagation and a critical factor involved is the cost of the culture medium which requires chemicals that are often very expensive.

The basic MS nutrients (Murashige and Skoog, 1962) is the most widely used media. However, the cost of tissue-culture based plant propagation is high in the developing countries, therefore there is a need to develop low cost micropropagation protocol to lower the cost of micropropagule to enable accessibility of tissue cultured planting material to farmers (Santana *et al.*, 2009). The shoot multiplication rate obtained in the low cost options was not less when compared to the other *in vitro* multiplication trails. (Ganapathi *et al.*, 1995) used tap water, commercial grade sugar and reduced the salt components in medium for banana plantlet production and achieved a maximum cost reduction of 31.2 %. Use of the media, culture vessel and low cost substitutes for mass propagation was successful in several other species (Sujatha and Chandran, 1997; Varshney *et al.*, 2000; Kodym and Arias, 2001 ; Kadota and Niim, 2004; Piatezak *et al.*, 2005; Hung *et al.*, 2006).

## Materials and Methods

### Plant materials

In the present study was to develop low cost tissue culture techniques in *Musa paradisiaca* L. var. Poovan and Monthan (Fig-1). The sword sucker of this plant were collected from National Research Centre for Banana (NRCB), Thogamalai road, Tiruchirappalli, Tamil Nadu, India. And these varieties are maintained in the college garden, PG and Research Department of Botany, Government Arts College, Ariyalur and were used as a source of mother plant in this, the sword suckers were used as a source material for *in vitro* studies.

### Low cost Banana Tissue culture Medium (LBTM) preparation

In the present study two tissue culture medium were used for the *in vitro* studies of *Musa paradisiaca* L. The first one was MS medium (Murashige and Skoog, 1962) and this medium composition was used as the control. The second one was low cost medium. (Table-1). Among the different low-cost alternative viz. macronutrients, micronutrients, iron source, vitamin source, carbon source, growth regulators and solidifying agent, The selected different low-cost nutrients and their combinations are standardized using sword sucker explants of *Musa paradisiaca* L. The standardized low cost nutrients are listed in the Table -1. This medium composition LBTM (low cost banana tissue culture medium) was used throughout the banana micropropagation.

### Inoculation:

Before starting inoculation all the required instruments such as media containing culture tubes, spirit lamp, sterile water, glass wares etc., were transferred to laminar air flow chamber and the platform surface of the chamber was swapped with 70 percent ethyl alcohol. Before inoculation, hands were rinsed with 70 percent alcohol. Then the explants were inoculated on the medium. Frequently forceps, scalpels, needles etc., were sterilized by dipping in 70 percent alcohol followed by flaming and cooling.

The inoculations were carried out in the vicinity of flame. The explants were taken out from beaker and at the same time the cotton plug of the culture tube was slightly opened in front of the spirit lamp flame, the explants have been put in and immediately covered with cotton plug. Hence, trimmed sword suckers were inoculated in vertical oriented plane on the low cost medium containing different combination and concentration of growth regulators (Table -1). Twenty culture tubes with single explants were assigned to each experiment and repeated thrice. The cultures were kept under 16 hours, light/day (2400 LUX) photo period at  $25 \pm 2^{\circ}\text{C}$ . The shoot multiplication was assessed after 4 weeks in culture by counting the proliferated shoots which attained the length of cm and above. The subsequent sub culture was made only on the medium which showed maximum shoot via multiple shoot induction.

### Effect of different growth regulators on shoot induction

In *Musa paradisiaca* L. sword suckers explants var. Poovan are supplemented with different combinations of Auxin and Cytokinin. Sword suckers explants were cultured on low cost medium (Table- 1), supplemented with various concentration of BAP and IBA (0.1, 0.2, 0.3, 0.4 and 0.5 mg/l) with 0.1 mg/l IAA for shoot induction (Table-2). The effect of growth regulators on the culture development response was studied and the effort was made to determine the optimal shoot growth combination.

### Effect of different growth regulators on multiple shoot induction

The well-developed shoots were sub cultured on low cost medium (Table-3), supplemented with various concentration of BAP (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) with 0.5 mg/l KIN for shoot multiplication. The effect

of growth regulators on the shoot multiplication response was studied and the effort was made to determine the optimal shoot multiplication combination.

### Root induction

Well-developed shoots were sub cultured into low cost root induction medium supplemented with different concentrations of IBA and GA<sub>3</sub> (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l) for root induction (Table -4). Rooting was observed at 10 to 15 days.

### Hardening

Plantlets with well-developed roots were removed from the culture tubes and transferred to greenhouse after washing their roots in running tap water, and grown in the mixture of auto claved sand+soil +vermi compost in 1:1:1 ratio in the plastic cups for 10 days and subsequently transferred to pots. The plants need 90-100% humidity and they were covered with plastic bags with perforation or holes (Table -5). After 20 days the plantlets in the plastic cups were transferred into soil.

### Potting mix

The *ex vitro* banana plantlets were transferred to the potting mixture for the growth and development of banana plants in the nursery stage. In the present study different potting mix viz: soil alone (control), soil+Press mud cake (PMC), Soil + Saw dust (SD), Soil +Rice husk (RH), Soil + Cattle dung manure (CDM),Soil + Coir pith (CP), Soil + Organic manure (OM), and Soil + Poultry manure (PM) were used for Banana nursery *ex vitro* propagation (Table -6).

### Statistical analysis

The well-developed cultures were examined periodically and morphological changes were recorded on the basis of visual observations. Whenever possible the effects of different treatments were quantified on the basis of percentage of cultures showing the responses per culture. The experimental design was completely randomized design (CRD) and factorial with Auxin and cytokinin as independent variables. All the experiments were repeated thrice. The data pertaining to frequencies of shoot induction, root induction, number of shoots was subjected to statistical analysis with Standard Deviations.

## Results

In the present study the MS nutrient composition was replaced by low cost medium (Table -1) i.e called LBTM (Low cost Banana Tissue culture Medium). The comparative Quantity per litre requirement was slightly increased. in compare with the individual requirement of convetinal MS medium composition (Table – 1).

### Effect of different growth regulators on Shoot induction frequency of var.Poovan

The trimmed surface sterilized sword sucker explant of *Musa paradisiaca* L. var. poovan were subjected to LBTM composition with different growth regulators. Among the different concentration of growth regulators BAP and KIN (0.1, 0.2, 0.3, 0.4 and 0.5 mg/l) with 0.1 mg/l IBA and IAA in the LBTM composition. The high shoot induction frequency was obtained in the combination of BAP 0.2 mg/l + IAA 0.1 mg/l (65.6±6.2) followed by KIN 0.2 mg/l + IAA 0.1 mg/l (60.6±6.8) respectively (Table- 2)

### Effect of different growth regulators on multiple shoot induction of var. Poovan

After 30 days old elongated shoot tip of banana are sub cultured in LBTM supplemented with different concentration of BAP and KIN (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) with IAA and IBA 0.5 mg/l for multiple shoot induction. Among the different growth regulators in the BAP 2.0 mg/l + IAA 0.5mg/l combination produced high frequency of shoots (18.3±3.7 ) followed by the combination of KIN 2.0 mg/l + IBA 0.5 mg/l (17.4±4.4 ) respectively (Table - 3).

### Effect of different growth regulators on Root induction of Poovan

After 41 days of old multiple shoot cultured were trimmed into single shoot tips using laminar air flow chambers. This shoot tips were transferred to rooting medium containing LBTM supplemented with different concentration of IBA (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) with GA<sub>3</sub> (0.5 mg/l) for root induction. Among the different concentration of IBA + GA<sub>3</sub> combination the 2.0 mg/l IBA + 0.5 mg/l GA<sub>3</sub> produced more number of roots (10.9 ± 2.6) followed by other combination. In all the IBA combination root was significantly produced in sub cultured banana shoot tips var. poovan. (Table- 4).

Table: 1 Low cost medium and conventional medium composition

Conventional MS medium (Murashige & Skoog composition, 1962)	Amount (mg/l)	Low cost Banana tissue culture medium composition (LBTM)	Amount (mg/l)
<b><u>Macro nutrients</u></b>		<b><u>Macro nutrients</u></b>	
Ammonium Nitrate (NH <sub>4</sub> NO <sub>3</sub> )	1650	Ammonium nitrate fertilizer	2200
Calcium chloride (CaCl <sub>2</sub> )	440	Calcium Chloride fertilizer	460
Potassium Nitrate (KNO <sub>3</sub> )	1900	Potassium Nitrate fertilizer	2100
Magnesium Sulphate (MgSO <sub>4</sub> )	370	Magnesium Sulphate fertilizer	420
Potassium dihydrogen Phosphate (KH <sub>2</sub> PO <sub>4</sub> )	170	Single super Phosphate	210
<b><u>Micro nutrients</u></b>		<b><u>Micro nutrients</u></b>	
Potassium iodide (KI)	0.83	Potassium Iodide(LR)	0.1
Boric oxide (H <sub>3</sub> BO <sub>3</sub> )	6.2	Power B-boran, Boric powder	15.0
Manganese Sulphate (MnSO <sub>4</sub> .4H <sub>2</sub> O)	22.3	Manganese Sulphate fertilizer	27.0
Zinc Sulphate (ZnSO <sub>4</sub> .7H <sub>2</sub> O)	8.6	Zinc Sulphate fertilizer	10.0
Sodium Molybdate (Na <sub>2</sub> MOO <sub>4</sub> .2H <sub>2</sub> O)	0.25	Adbor powder	0.50
Copper Sulphate (CuSO <sub>4</sub> . 5H <sub>2</sub> O)	0.025	Chelated fertilizer	0.025
Cobalt chloride (COCl <sub>2</sub> )	0.025	Grandular/ powder	0.025
<b><u>Iron Nutrient</u></b>		<b><u>Iron Nutrient</u></b>	
Ethylene diamine tetra acetic acid (EDTA)	1.9	Ethylene diamine tetra acetic acid (EDTA)	2.1
Ferrous Sulphate (FeSO <sub>4</sub> .7H <sub>2</sub> O)	1.39	Ferrous Sulphate fertilizer	1.6
<b><u>Vitamins</u></b>		<b><u>Vitamins</u></b>	
Myo Inositol	100	Becosules B-complex Tablets containing	15.0
Glycine	2	Thiamine	
ThiamineHcl	0.1	Riboflavin	
Nicotinic acid	0.5	Pyridoxine HCl	
Pyridoxine Hcl	0.5	Ascorbic acid	
		Biotin	
		Folic acid	
		Calcium pantothenate	
		Niacinamide	
<b><u>Growth regulators *</u></b>		<b><u>Growth regulators*</u></b>	
IAA	0.1	IAA	0.1
2, 4-D	0.1	2, 4-D	0.1
NAA	0.1	NAA	0.1
IBA	0.1	IBA	0.1
Kinetin	0.1	Kinetin	0.1
BAP	0.1	BAP	0.1
GA <sub>3</sub>	0.1	GA <sub>3</sub>	0.1
<b><u>Carbon source</u></b>		<b><u>Carbon source</u></b>	
Sucrose	30 (g)	White refined sugar (Table sugar)	30 (g)
<b><u>Solidifying agent</u></b>		<b><u>Solidifying agent</u></b>	
Agar - Agar	8(g)	Agar Agar (AR)	8 (g)

**Table : 2 Effect of BAP and Kin in combination of (0.1 mg/l) IAA and IBA supplemented with LBTM (low cost banana tissue culture medium) on shoot induction of *Musa paradisiaca* L. Var. Poovan using sword suckers explants.**

Concentration of growth regulators (mg/l)	% of response	Shoot induction frequency
<b>BAP+ IAA</b>		
0.1+0.1	88.1±1.8	42.1±1.4
<b>0.2+0.1</b>	<b>92.6±7.1</b>	<b>65.6±6.2</b>
0.3+0.1	82.4±5.6	40.4±5.6
0.4 +0.1	75.1±2.4	55.1±2.0
0.5+0.1	80.5±3.8	52.5±2.8
<b>BAP+ IBA</b>		
0.1+0.1	80.0±4.0	60.0±4.0
0.2+0.1	74.1±4.8	52.0±3.8
<b>0.3+0.1</b>	<b>82.6±3.8</b>	<b>60.1±2.8</b>
0.4 +0.1	85.2±3.6	44.2±0.6
0.5+0.1	84.8±2.8	38.8±2.9
<b>KIN +IAA</b>		
0.1+0.1	72.4±1.8	38.4±1.8
<b>0.2+0.1</b>	<b>90.6±6.2</b>	<b>60.6±6.8</b>
0.3+0.1	86.8±1.2	44.8±1.8
0.4 +0.1	82.8±3.8	38.8±3.8
0.5+0.1	78.6±2.0	55.6±2.0
<b>KIN +IBA</b>		
0.1+0.1	70.8±6.4	35.8±6.0
0.2+0.1	85.8±7.1	44.8±7.2
0.3+0.1	68.4±1.6	34.4±1.8
<b>0.4 +0.1</b>	<b>86.2±2.8</b>	<b>38.2±2.8</b>
0.5+0.1	76.6±1.9	25.6±1.0

*Data presented as the mean value ± standard error after 30 days of culture from four independent experiments each with 10 replicates..*

**Table : 3 - Effect of BAP and KIN in combination of (0.5 mg/l) IAA and IBA supplemented with LBTM (low cost banana tissue culture medium) on multiple shoot induction of *Musa paradisiaca* L. Var. Poovan using sword suckers explants.**

Concentration of Growth regulators (mg/l)	% of response	No. of Shoots
<b>BAP+IAA</b>		
1.0+0.5	80.2±1.8	15.5±1.7
1.5+0.5	74.2±3.9	14.3±4.6
<b>2.0+0.5</b>	<b>94.2±3.4</b>	<b>18.3±3.7</b>
2.5 +0.5	88.2±6.1	12.1±6.3
3.0+0.5	84.2±7.3	10.8±5.3
<b>BAP +IBA</b>		
1.0+0.5	88.5±3.8	9.1±3.7
1.5+0.5	83.1±3.2	10.4±3.8
<b>2.0+0.5</b>	<b>95.8±2.4</b>	<b>15.6±4.2</b>
2.5 +0.5	82.0±2.2	10.0±5.6
3.0+0.5	80.0±1.3	8.1±1.8
<b>KIN +IAA</b>		
1.0+0.5	75.2±3.5	12.5±3.2
<b>1.5+0.5</b>	<b>88.6±3.2</b>	<b>17.2±4.8</b>
2.0+0.5	78.4±4.2	14.3±4.8
2.5 +0.5	81.6±3.2	14.1±3.6
3.0+0.5	82.2±4.8	13.6±4.2
<b>KIN +IBA</b>		
1.0+0.5	84.1±2.8	10.5±3.1
1.5+0.5	88.8±3.1	17.0±3.8
<b>2.0+0.5</b>	<b>95.2±4.1</b>	<b>17.4±4.4</b>
2.5 +0.5	89.3±4.8	10.2±2.6
3.0+0.5	78.8±3.6	9.47±3.6

*Data presented as the mean value ± standard error after 30 days of culture from four independent experiments each with 10 replicates..*

**Table : 4. Effect of LBTM supplemented with different concentration of IBA with GA<sub>3</sub> (0.5 mg/l) on root induction frequency of *Musa paradisiaca* L. Var. Poovan**

Concentration of growth regulators (mg/l)	Root induction frequency (Mean $\pm$ SD)	No. of roots (Mean $\pm$ SD)
<b>IBA + GA<sub>3</sub></b>		
0.5+0.5	12.8 $\pm$ 1.8	9.5 $\pm$ 3.2
1.0+0.5	15.8 $\pm$ 2.5	4.3 $\pm$ 2.1
1.5+0.5	13.6 $\pm$ 2.1	4.6 $\pm$ 1.8
<b>2.0+0.5</b>	<b>18.4<math>\pm</math> 3.6</b>	<b>10.9<math>\pm</math>2.6</b>
2.5+0.5	14.8 $\pm$ 1.7	10.5 $\pm$ 3.2
3.0+0.5	10.8 $\pm$ 2.5	14.3 $\pm$ 2.1

*Data presented as the mean value  $\pm$  standard error after 30 days of culture from four independent experiments each with 10 replicates..*

### Hardening

The plantlets having sufficient root and shoot system were taken out from the culture were washed under running tap water to remove the solidifying agent. The plantlets were transferred to poly cups containing autoclaved sand, soil and vermi compost (1:1:1) mixture and maintained in the green house conditions.

Among the different low cost autoclaved compost mixture were also tried for hardening (Table – 5). Among the different composition mixture autoclaved sand + garden soil + vermi culture showed high survival percentage 78  $\pm$  1.2 respectively (Table – 5).

**Table - 5 Effect of different low cost compost mixture for hardening on *Musa paradisiaca* L.**

Compost mixture	Number of plantlets transferred	Survival (%)	Average shoot weight (cm)	Average Leaves plantlet
Autoclaved sand, Vermicompost +Vermiculite (1:1:1)	10	65 $\pm$ 0.16	5.2 $\pm$ 1.0	2.8 $\pm$ 0.5
Autoclaved sand Garden soil +Vermiculite (1:1:1)	10	78 $\pm$ 1.2	7.3 $\pm$ 0.6	3.2 $\pm$ 0.4
Autoclaved sand Humus + Vermiculite (1:1:1)	10	85 $\pm$ 1.71	8.1 $\pm$ 0.2	3.8 $\pm$ 0.3

*Data presented as the mean value  $\pm$  standard error after 30 days of transfer.*

**Banana *in vivo* propagation using low cost potting mix**

The well developed *ex vitro* banana shoots (var. poovan) were transferred to *in vivo* condition. Using different potting mix and several parameters like height, leaf length, No.of leaves and No.of primary roots were recorded. Among these in the potting mix

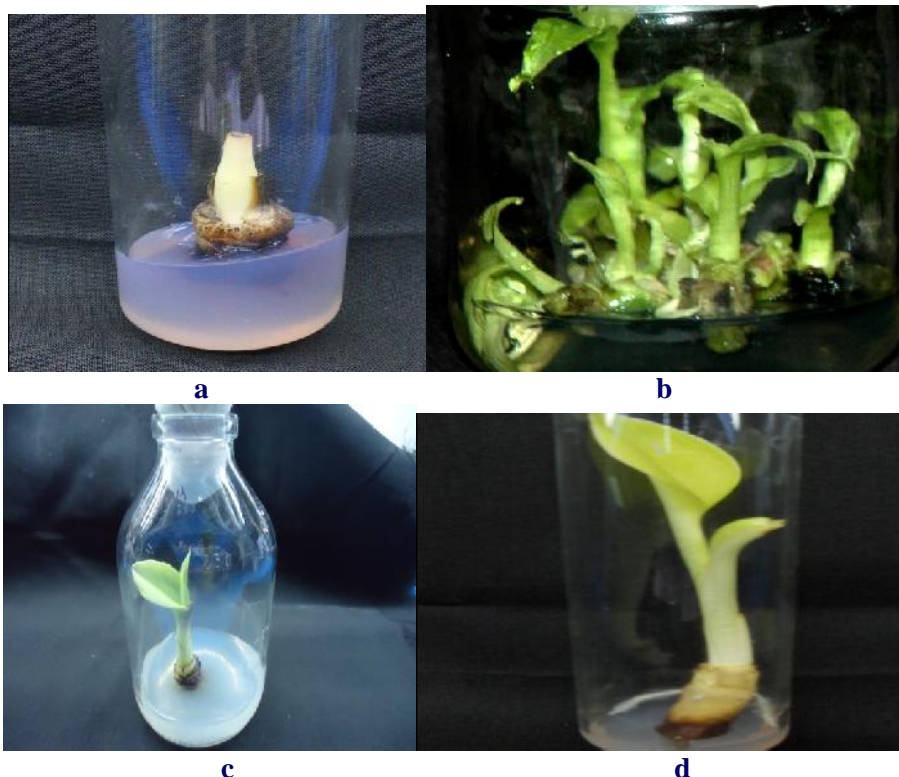
(Soil+PM) plantlets showed maximum frequency(18.5; 17.5 cm) in 30 days compare to the other potting mix. Likewise, leaf length (22.1cm) and No.of leaves (8) in the same potting mix (Soil+PM) was achieved. In all the other potting mix (Soil+OM) more primary roots than the other potting mix (Table – 6).

**Table : 6 Effect of different low cost potting mixture for the *In vivo* propagation of *Musa paradisiaca* L. Var. Poovan**

Potting medium	Height (cm)	Leaf length (cm)	No of leaves	No. of primary roots
Soil alone (control)	9.6	10.8	5	6
Soil + PMC	16.2	18.8	6	15
Soil + SD	8.9	10.2	4	4
Soil + RH	12.6	14.6	5	8
Soil + CDM	10.9	13.2	6	7
Soil + CP	11.5	14.0	7	12
Soil + OM	12.2	12.2	6	10
Soil + PM	8.5	22.1	8	8

PMC = Press mud cake, SD= Saw dust, RH= Rice Husk, CDM= Cattle Dung Manure, CP= Coir Pith, OM= Organic Manure, PM= Poultry Manure

**Effect of LBTM supplemented with different concentration of growth regulators on micropropagation of *Musa paradisiaca* L. var. poovan using sword sucker explants.**







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- a- Shoots induction
- b- Multiple shoot
- c- Shoot elongation
- d- Root induction
- e- Hardening

## Discussion

In this present investigation, effect of different concentrations of BAP+IAA on shoot induction, and shoot multiplication were investigated. The shoot induction and multiplication with the implementation of different concentration of BAP along with LBTM were presented in (Table – 2&3). A part from the influence of genotypes, shoot proliferation rate and elongation are affected by cytokinin types and their concentration. Adenine based cytokinins are used in several *Musa* sp. For *in vitro* propagation, BAP is the most commonly prepared cytokinin (Vulsteke, 1989).

The concentration of exogenous cytokinin appears to be the main factor affecting multiplication of shoot. For example, Wong (1986) stated that when 11.1  $\mu\text{M}$  BAP is supplemented in the medium, each of the explants produces as average of shoots, while increasing BAP concentration of 65.6 shoot induction frequency in per explants respectively. However, the optimum recommended BAP concentration is 20 $\mu\text{M}$  for banana micropropagation.

*In vitro* propagation using benzylaminopurine (BAP) is the most commonly preferred cytokinin, Frequency of shoot induction was increased of subculture in the same medium. In early studies, optimum BAP concentrations were found to be by Cronauer and Knkorian 1984; and Jarret *et al.*, 1986; and 20 $\mu\text{M}$  by Vuyslreke 1989, Wong 1986 stated that 44.4  $\mu\text{M}$  BAP reduced shoot multiplication. Arinative *et al.*, 2000 stated that shoot proliferation is cultivar dependent.

Similar results obtained using BAP in Banana propagation Var. poovan 65.6% shoot induction respectively.

Likewise, low cost alternative was used in several plants. the shoot multiplication rate obtained in the low cost options was not less when compared to the other *in vitro* multiplication trails. (Ganapathi *et al.*, 1995) used tap water, commercial grade sugar and reduced the salt components in medium for banana plantlet production and achieved a maximum cost reduction of 31.2 %. Use of the media, culture vessel and low cost substitutes for mass propagation was successful in several other species (Sujatha and Chandran, 1997; Varshney *et al.*, 2000; Kodym and Arias, 2001 ; Kadota and Niim, 2004; Piatezak *et al.*,2005; Hung *et al.*, 2006).

In present study root initiation and development was observed in all the media containing different concentrations of NAA, and also in the conventional medium and low cost media containing a combination of NAA and GA<sub>3</sub>. Bekeet and Saker (1999) reported the superiority of NAA and IAA and IBA in the *in vitro* rooting of banana plantlets cv. William, Grande Naine and Maghraby while Ahsani *et al.*, 1998 reported that the best response was achieved in hormone free Ms media for table banana (*Musa sapientum*). The present finding of root induction in hormonal combinations confirms the findings of Baby *et al.*, 1997 who achieved 100% rooting in low cost media supplemented with various combination of NAA and GA<sub>3</sub>.

Similarly in Banana var. Poovan *ex vitro* plantlets are tried different potting mix. In several authors reports. The use of organic matter such as animal manures, human waste, food wastes, yard wastes, sewage sludges and composts has long been recognized in agriculture as beneficial for plant growth and yield and the maintenance of soil fertility. Reddy and Reddy 1999, reported significant increases in micronutrients in field soils after vermicomposting applications compared to those in soils treated with animal manures. In other experiments, amounts of soil nitrogen increased significantly after incorporating Vermi composts into soils.

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